Kidney International, Vol. 25 (1984), pp. 619–628

Extracellular fluid expansion and autoregulation in nephrotoxic serum nephritis in rats

TENEI SAKAI, FRIEDRICHS H. HARRIS, JR., DONALD J. MARSH, CLEAVES M. BENNETT, and RICHARD J. GLASSOCK
with the technical assistance of CHRISTINE MARTIN, BENNETT KAYSER, and CHARLES CHUNG

Department of Physiology and Biophysics, University of Southern California School of Medicine, Los Angeles, and Department of Medicine, University of California Los Angeles School of Medicine, and Harbor-University of California Los Angeles Medical Center, Torrance, California

Extracellular fluid expansion and autoregulation in nephrotoxic serum nephritis in rats. Previous studies of the early autologous phase of bilateral nephrotoxic serum nephritis (NSN) in rats showed that whole kidney and single nephron glomerular filtration rate (GFR) were maintained at normal levels despite a 60% reduction in the product of surface area and hydraulic permeability (Kf). Factors responsible for this compensation were an increase of net ultrafiltration pressure, due primarily to an increased glomerular capillary pressure (P_{GC}). This study was designed to investigate some possible causes of the compensation. Rats with bilateral NSN and normal GFR had an increased extracellular fluid volume (ECFV) 2 weeks after induction of NSN; control subjects did not change. To determine whether this ECFV expansion was responsible for triggering the compensation, we developed a unilateral NSN model with one diseased and one normal kidney. Unilaterally diseased rats did not experience an increase of ECFV. Values of Kf were 0.069 ± 0.012 nl sec^{-1} mm Hg^{-1} in control subjects, 0.037 ± 0.005 in bilateral NSN, and 0.043 ± 0.006 in unilateral NSN. The elevation in P_{GC} was the same in unilateral NSN as in bilateral NSN subjects and the same was true for the hydrostatic pressure difference across glomerular capillaries ($\Delta\bar{P}$). Furthermore, in paired measurements on both kidneys of rats with unilateral NSN, P_{GC} was significantly higher in the unilaterally diseased kidney than in the nondiseased kidney; sham control subjects had no difference. These results are interpreted to indicate that the signal that causes elevation of net ultrafiltration pressure is not a consequence of a systemic effect of NSN, but arises within the diseased kidney itself. To determine whether that signal involved some change in the mechanisms mediating autoregulation measurements were made of the response of whole kidney GFR and RBF to acute changes in arterial BP. Control rats and rats with NSN autoregulated both GFR and blood flow equally well. Tubuloglomerular feedback was studied by microperfusing loops of Henle and measuring proximal stop-flow pressure and early proximal flow rate. Stop-flow pressure was 4.0 mm Hg higher in rats with NSN at a loop perfusion rate of 10 nl/min, approximately the same difference that was found by direct measurement of P_{GC}, but the sensitivity of response to changes in perfusion rate was the same in NSN as in control subjects. Finally, end proximal tubule flow rate was higher in NSN than in control subjects reflecting decreased proximal reabsorption. Thus, a normal feedback mechanism receives a signal that should cause afferent arteriolar constriction in NSN rats. Afferent arteriolar constriction would lead to a fall in P_{GC}, so the signal that causes the observed increase must arise elsewhere than in the mechanisms that mediate autoregulation, but the identity of the signal remains unknown.

L'expansion volémique extracellulaire et l'autorégulation dans la néphrite sérique néphrotoxique chez des rats. Des études antérieures de la phase autologue précoce de la néphrite sérique néphrotoxique bilatérale (NSN) chez des rats ont montré que les débits de filtration glomérulaire (GFR) du rein entier et des néphrons individuels étaient maintenus à des niveaux normaux malgré une réduction de 60% du produit de la surface et de la perméabilité hydraulique (Kf). Les facteurs responsables de cette compensation étaient une augmentation de la pression d'ultrafiltration nette due essentiellement à une augmentation de la pression capillaire glomérulaire (P_{GC}). Ce travail a été entrepris pour étudier quelques causes possibles de cette compensation. Des rats avec une NSN bilatérale et une GFR normales avaient une augmentation du volume extra-cellulaire (ECFV) 2 semaines après l'induction de la NSN: les contrôles n'avaient pas de modification. Afin de déterminer si cette expansion de l'ECFV était responsable du déclenchement de la compensation, nous avons développé un modèle de NSN unilatérale avec un rein normal et un rein malade. Les rats avec une atteinte unilatérale n'ont pas eu d'augmentation de l'ECFV. Les valeurs de K_f étaient de 0,069 ± 0,012 nl sec^{-1} mm Hg^{-1} chez les contrôles, 0,037 ± 0,005 chez les NSN bilatérales, et 0,043 ± 0,006 chez les NSN unilatérales. L'élévation de P_{GC} était identique chez les NSN unilatérales et les NSN bilatérales, et ceci était également vrai pour les différences de pressions hydraustatiques à travers les capillaires glomérulaires ($\Delta\bar{P}$). En outre, lors de mesures appariées sur les deux reins des rats avec une NSN unilatérale, P_{GC} était significativement plus élevée dans le rein malade unilatéral que dans le rein sain; les sujets contrôles n'avaient pas de différence. Ces résultats tendent à indiquer que le signal qui est à l'origine de l'élévation de la pression d'ultrafiltration nette n'est pas une conséquence d'un effet systémique de la NSN, mais provient du rein malade lui même. Afin de déterminer si le signal mettait en jeu une modification des mécanismes médiant l'autorégulation, des mesures de la réponse de la GFR et du flux sanguin rénal du rein entier ont été effectuées lors de modifications aiguës de la pression artérielle. Les rats contrôles et les rats atteints de NSN autorégulaient leur GFR et leur flux sanguin aussi bien. Le feedback tubuloglomérulaire a été étudié en microperfusant des anses de Henlé et en mesurant la pression d'occlusion proximale et le débit dans le proximal précoce. La pression d'occlusion était 4,0 mm Hg plus élevée chez les rats atteints de NSN pour une perfusion de l'anse de 10 nl/min, approximativement la même différence qui était trouvée par mesure directe de P_{GC}, mais la sensibilité de la réponse aux modifications de vitesse de perfusion était la même chez les NSN et les contrôles. Finalement, le débit liquidien dans la fin du tubule proximal était plus élevé chez les NSN que chez les sujets contrôles, ce qui reflète une diminution de la réabsorption proximale. Ainsi, un mécanisme de feedback normal reçoit un signal qui pourrait entraîner une constriction de l'artériole afférente chez les rats NSN. La constriction artériolaire afférente pourrait entraîner une diminution de P_{GC}, de sorte que le signal qui est à l'origine de l'augmentation observée doit se produire à un autre endroit que les mécanismes qui médient l'autorégulation, mais l'identité du signal reste inconnue.

An accelerated form of the autologous phase of nephrotoxic serum nephritis (NSN) can be induced in rats by the injection of

Received for publication February 17, 1983
and in revised form August 3, 1983

rabbit anti-rat glomerular basement membrane antibody in suitably sensitized animals [1, 2]. The severity of the lesion can be controlled, and it is routinely possible to observe normal glomerular filtration rate (GFR) despite histological evidence of glomerular injury. Maddox et al [3] used this model and found that nephritic animals with normal GFR have increased net ultrafiltration pressure due primarily to elevated glomerular capillary pressure (P_{GC}). The surface area-filtration permeability product (Kf) in these nephritic rats was approximately one-half of that found in normal rats.

The GFR is the product of Kf and mean net ultrafiltration pressure. The normal GFR of NSN results from the fact that these two variables change in opposite directions. Some change in arteriolar resistances must cause the change in net ultrafiltration pressure. The change could be a decrease in afferent arteriolar resistance alone or in concert with an increase in efferent arteriolar resistance. Dilatation of the afferent arteriole would by itself increase P_{GC} and increase renal blood flow (RBF). Maddox et al [3] reported a slight increase in RBF, but the increase was not significant. A decrease of about 20% in afferent arteriolar resistance would be necessary to account for the observed increase in P_{GC}; a resistance change of this magnitude would increase RBF about 12%. If the decrease of arteriolar resistance were combined with an increase of efferent arteriolar resistance, the increase in P_{GC} would be reinforced, but the effect on total renal vascular resistance would be offset, and there would be little or no change in RBF. If the dilatation of the afferent arteriole is accompanied by a constriction of the efferent arteriole, the reduction of the afferent arteriolar resistance necessary to cause the observed elevation of P_{GC} would be less than the 20% reduction required if the afferent arteriole is the sole site of change. The methods available for the measurement of arteriolar resistances in the renal circulation can measure resistance changes of these magnitudes when intra-animal comparisons can be made, but they are less suitable when the circumstances dictate comparisons between groups of animals. Unfortunately, the change we wish to study develops over a 2-week period, and comparisons in single animals are not possible. We wish to determine the cause of the adaptation. Because we cannot expect to infer the cause from direct measurements of arteriolar resistances, we have sought to examine the operation of mechanisms that might affect the resistances.

In this study we tested two possibilities: that expansion of the extracellular fluid volume (ECFV) generates an extrarenal signal or that a change of the operating characteristics of tubuloglomerular feedback is responsible. Maddox et al [3, 4] studied rats with NSN 10 to 14 days after the disease was induced. In preliminary studies we found that nephritic rats with disease of the same duration had an expanded ECFV compared to control litter mates [5]. There are several reports [6, 7] that ECFV expansion reduced the ability of feedback to modulate GFR when flow rate of tubular fluid past the macula densa was increased. A corollary to this result is that GFR could increase at normal macula densa flow rates and could restore GFR to normal if macula densa flow rates fall. To test this possibility we developed and studied a unilateral model of NSN [8], in which ECFV is not increased because of the function of the normal kidney. The results with this model are the same as with bilateral NSN, that is, elevation of P_{GC}.

Even though ECFV expansion does not seem to be a necessary requirement for hemodynamic adaptations to NSN, other factors, known or unknown, could affect the operation of tubuloglomerular feedback. We therefore studied the known modalities of blood flow and GFR regulation by assessing whole kidney autoregulation and the response to perfusion of loops of Henle. No differences were found between nephritic and control rats. The cause of the dilatation must be sought elsewhere.

Methods

Animals. Male Sprague-Dawley and Wistar-Munich rats weighing 156 to 460 g were used throughout these studies. The Wistar-Munich rats were originally obtained from Drs. Barry Brenner and David Maddox of the University of California at San Francisco, and later from a breeding colony established in our laboratory. Except as otherwise specified, prior to the hemodynamic measurements the rats were maintained on standard laboratory chow containing sodium.

Preparation and characterization of the heterologous anti-rat glomerular basement membrane antibody (anti-GBM Ab)

New Zealand white rabbits were immunized repeatedly with ultrasonicated, insoluble rat GBM incorporated in complete Freund's adjuvant (CFA) as previously described [3]. Rabbit IgG (RaIgG) was isolated from normal, non-immune and immune rabbit sera by DEAE cellulose chromatography. Anti-GBM Ab content was determined by a paired-label method previously described [9].

Production of models of nephrotoxic serum nephritis (NSN) and controls

Bilateral NSN. Rats were preimmunized once in each rear footpad with 2.0 mg of normal rabbit gamma globulin (Cohn Fraction II, Miles Laboratory-Research Products, Elkhart, Indiana) incorporated in 0.5 ml of CFA (9 ml of Baylol F, 1 ml Arlacel 83, and 6 mg Mycobacterium Tuberculosis, H37Ra, Difco Laboratories, Detroit, Michigan). Two days later the rats were infused via a tail vein with immune RaIgG calculated to contain 50 to 100 μg of anti-GBM Ab. After the infusion the rats were permitted free access to fluids and food until the study was begun. Twenty-four-hour urinary protein excretion (Kingsbury-Clark method; normal values for rats, 3.2 ± 1 mg/24 hr) was measured immediately prior to the hemodynamic studies. Formalin-fixed and snap-frozen specimens of the right and left kidney were obtained at sacrifice 5 to 15 days after infusion of anti-GBM Ab. Light microscopy (hemotoxylin and eosin and periodic acid-Schiff stains) and immunofluorescent microscopy were performed on these specimens (see below).

Unilateral NSN. The animals were preimmunized with RaIgG, as described above, and 2 days later were anesthetized with sodium pentobarbital (3 mg/100 g body weight); the left and right kidneys were exposed via a midline abdominal incision. Using a technique modified from that described by Hoyer, Mauer, and Michael [8], the left kidney was perfused with immune RaIgG calculated to contain 50 to 100 μg of anti-GBM Ab. In brief, the left renal artery (RA) and vein (RV) and aorta, and right RA were mobilized. The spermatic and adrenal vessels were isolated and ligated. Loose ligatures were placed around the aorta proximal and distal to the left renal artery, around the right RA and left RV. At approximately the same

time, the ligatures around the aorta were tightened so as to occlude the vessels. Immediately thereafter, a 25-gauge needle was inserted into the aorta just proximal to the distal ligature and the tip was advanced to the level of the left RA. Heparinized physiological saline was infused via an infusion pump (Harvard, Harvard Apparatus, Edenridge, Kent, United Kingdom) at 0.8 ml/min. The left renal vein was subsequently pierced with a 28-gauge needle. Perfusion was continued until the effluent from the renal vein was clear. The ligature on the right RA was then tightened, and using a three-way valve, RaIgG containing 50 to 100 μg of anti-GBM Ab in 0.4 ml of saline was infused manually over 30 sec into the left RA. The renal-venous effluent was blotted carefully during this and subsequent procedures. Three minutes after the conclusion of the infusion, the left kidney was again perfused with 2.0 ml of saline at a rate of 0.8 ml/min. The aortic perfusion needle was then removed and the aortic and renal venous puncture sites sealed with a small piece of sterile microcrystalline collagen hemostats (Avicon Inc., Puerto Rico). All ligatures on the aorta and left RV were then removed. Five minutes later the ligature on the right RA was removed. In no case was blood flow interrupted to either kidney for more than 15 min. The abdominal incision was then closed. Rats were returned to metabolic cages and allowed free access to water and fluid as was the method used for bilateral NSN rats. At the time of sacrifice, 5 to 15 days after the infusion of anti-GBM Ab, specimens of the right and left kidney were obtained in formalin and snap-frozen state for light and immunofluorescence microscopy.

Controls. Normal Wistar-Munich rats were preimmunized with normal rabbit gamma globulin as described above, but instead of receiving anti-GBM Ab, they were given a comparable amount of non-immune RaIgG intravenously (NSN control rats) or via the left renal artery as described above (unilateral sham control rats). At sacrifice, 5 to 15 days after infusion, specimens were also obtained for light and immunofluorescence microscopy as described below. In a further set of normal animals hemodynamic measurements were made following the acute infusion of homologous plasma (approximately 10% of the body weight) to calculate absolute values of Kf. In a separate set of observations animals with unilateral NSN and unilateral sham control rats underwent P_{GC} measurements in both the left and right kidneys.

Measurement of extracellular fluid volume (ECFV)

Twenty-six male Sprague-Dawley rats weighing 314 to 460 g were divided into three groups: group 1, animals with bilateral NSN (N = 10); group 2, unilateral NSN (N = 8); and group 3, normal controls (N = 8). Whole animal GFR and ECFV were measured before and 2 weeks after the induction of nephritis. GFR and ECFV were estimated by following the time course of tritiated inulin and sulfate-35, respectively, following single intracardiac injections.

After simultaneous intracardiac injections of isotopes under ether anesthesia, blood was collected from the tail vein in previously weighed heparinized hematocrit tubes at 30, 60, 90, and 120 min. The tubes were weighed again, the hematocrit was measured, and the weight of the plasma was calculated. The plasma water volume was assumed to be 0.93 times plasma weight. Urine samples were also collected by the use of metabolic cages after 30, 60, 90, and 120 min. The collection funnel was washed with phosphate-buffered saline (PBS) solution and was included in each collection. The volume of isotope injected was determined by weighing the syringe before and after the injection. The amount of ^3H and ^{35}S administered averaged 1.4 and 0.7 μCi, respectively. The isotopes in the plasma, urine, and standard were measured by the use of a liquid scintillation counter (LS-333, Beckman Instruments, Inc., Fullerton, California). Appropriate corrections were made for dual labeling, quenching, and background, to calculate disintegration per minute.

Calculations

GFR was calculated as K_IQ/A where K_I is the slope of inulin disappearance, Q is the total amount injected, and A is the Y intercept, that is, inulin concentration at zero time. ECFV was measured as the volume of distribution of ^{35}S (VD) and was calculated from the equation: VD = (Total ^{35}S injected − Total urine ^{35}S/plasma water ^{35}S). VD was expressed as milliliters per 100 g of body weight and GFR as milliliters per minute. The VD was calculated for each time interval in which a urine collection was obtained. The time intervals in which no urine could be collected or in which there was a greater than 10% variation from the previous time interval were discarded. The remaining time intervals were then averaged for a final VD. At least two of the four time intervals were required. Recovery experiments that involved pouring rat urine with ^{35}S into a metabolic cage and washing the collecting funnel with PBS resulted in an isotope recovery rate of 90% + 2. There was no difference in recovery rate as long as more than 10 ml of PBS were used. The urine count of ^{35}S for each time interval was therefore divided by 0.9 for the total urine count.

Micropuncture experiments

Experiments were performed in adult Wistar-Munich rats allowed free access to food and water before study. Rats were anesthetized with Inactin (100 mg/kg) and prepared for micropuncture as described previously [3]. Sixty minutes before the micropuncture, rats received an intravenous infusion of isotonic sodium chloride containing 10% inulin at the rate of 0.02 ml/min. Mean femoral arterial pressure ($\bar{AP}$) was monitored by means of an electronic transducer (Statham Instruments, Inc., Oxnard, California) connected to a direct-writing recorder. After a 60-min equilibration period, exactly timed samples of fluid were collected from randomly selected proximal tubules for the determination of flow rate and inulin concentration and for calculation of SNGFR. Collections were made by holding an oil droplet in position distal to the puncture site. Coincident with these tubule fluid collections, femoral arterial blood samples (100 μl) were obtained for the determination of hematocrit and plasma inulin concentration.

Hydraulic pressures were measured in single glomerular capillaries of surface glomeruli using a continuous recording, servonulling micropipette transducer. Direct measurements of hydraulic pressure in single glomerular capillaries (P_{GC}), proximal tubules (P_T), efferent arterioles (P_E), and second or third-order peritubular capillaries (P_C) were recorded in each rat. Mean values for each animal were derived from measurements in three to five individual structures of each type. To obtain estimates of colloid osmotic pressure (π) of plasma entering and leaving glomerular capillaries, protein concentrations in femo-

ral arterial and efferent arteriolar blood plasma were measured as previously described. Colloid osmotic pressures (π) for control and experimental rats were calculated from these measured values by using the equation $\pi = 1.63\ c + 0.294\ c^2$ (c = total protein concentration in g/dl). For plasma with albumin/globulin ratios of less than 0.8, the equation $\pi = 2.24\ c + 0.180\ c^2$ was utilized [10]. These estimates of pre- and postglomerular protein concentration permit calculation of single nephron filtration fraction (SNFF) and initial glomerular plasma flow (GPF). From direct measurements of the decline in pressure along single afferent and efferent arterioles and the estimates of blood flow through these vessels, vascular resistances to blood flow were calculated (see below).

Analytical. The volume of tubule fluid collected from individual nephrons was estimated from the length of the fluid column in a constant-bore capillary tube of known internal diameter. Concentration of inulin in tubule fluid was measured in duplicate by the microfluorescence method of Vurek and Pegram [11]. Inulin concentration and arterial plasma was determined by the macroanathrone method [12]. Protein concentration in efferent arteriolar and femoral arterial blood plasmas was determined in duplicate with an ultramicro-colorimetric method using the micro-adaptation of the method of Lowry et al [13].

Calculations

Single nephron glomerular filtration rate:

$$SNGFR = (TF/P)_{In} \times V_{TF} \quad (1)$$

where $(TF/P)_{In}$ and V_{TF} are referred to transtubular inulin concentration ratio and tubular fluid flow rate, respectively.

Single nephron filtration fraction:

$$SNFF = 1 - C_A/C_E \quad (2)$$

where C_A and C_E denote afferent and efferent arteriolar protein concentrations, respectively.

Afferent arteriolar glomerular plasma flow rates:

$$GPF = SNGFR/SNFF \quad (3)$$

Afferent arteriolar blood flow rate:

$$GBF = GPF/(1 - Hct) \quad (4)$$

where Hct is the arterial hematocrit.

Efferent arteriolar blood flow rate:

$$EGBF = GBF - SNGFR \quad (5)$$

Single afferent arteriole resistance:

$$R_a = 7.962 \times 10^{10}\ (A\bar{P} - P_{GC})/GBF \quad (6)$$

where the factor 7.962×10^{10} provides resistance in units of dynes $\times$ seconds $\times$ centimeter/s-5 where $A\bar{P}$ and P_{GC} are expressed in millimeters of mercury and GBF in nanoliters per minute.

Single efferent arteriole resistance:

$$R_E = 7.962 \times 10^{10}\ (P_{GC} - P_C)/EGBF \quad (7)$$

Total arteriolar resistance:

$$R_T = R_A + R_E \quad (8)$$

Net ultrafiltration pressure at the afferent end of the glomerular capillary:

$$P_{UFA} = P_{GC} - P_T - \pi a \quad (9)$$

Net ultrafiltration pressure at the efferent end of the glomerular capillary:

$$P_{UFE} = P_{GC} - P_T - \pi e \quad (10)$$

Mean transglomerular hydraulic pressure difference:

$$\Delta\bar{P} = P_{GC} - P_T \quad (11)$$

Whole kidney GFR autoregulation study (Sprague-Dawley rats, bilateral NSN)

After tracheostomy, a jugular vein was catheterized and a 10% inulin solution in isotonic saline was infused. A priming dose of about 0.5 ml was given over 10 min and thereafter a steady infusion (1.2 ml/hr) was maintained for at least 45 min before measurements were made. A left flank incision was made and the left kidney was placed in a cup (Lucite®). The surface of the kidney was covered with transparent wrap (Saran-wrap®) and the ureter was cannulated with PE 10 tubing. A section of the aorta between the two renal arteries was gently dissected free with care taken to avoid rupturing the thoracic duct. An aortic clamp was placed cephalad to the left renal artery to vary the arterial perfusion pressure of the left kidney. The systemic arterial pressure was elevated by bilateral occlusion of the carotid arteries. Urine samples were collected with glass capillary tubes over 3- to 5-min periods. Triplicate or quadruplicate collections were made at both high arterial pressure of around 140 mm Hg (carotid occlusion) and at a low pressure of 105 mm Hg (aortic constriction). Urine flow rates were determined gravimetrically. Blood samples were obtained before and after each period of urine collections.

Renal blood flow autoregulation (Sprague-Dawley rats, bilateral NSN)

A separate group of animals was used for this study. A left flank incision was made and the left renal artery was gently freed from its surrounding tissue. Whole kidney RBF measurements were made by fitting the renal artery with a small lumen size (2.0 mm circumference) flow transducer (Model EP 102, Carolina Medical Electronic Inc.). The flow transducer was connected to an electromagnetic flow meter (Model BL-610, Biotronex Lab. Inc.). Calibration of the flow meter was done by pumping heparinized blood from donor rats at known rates through an excised piece of carotid artery. After the surgical preparations, the RBF and arterial pressure were allowed to stabilize for at least 20 min. In each experimental run, arterial pressure was first elevated by bilaterial carotid artery occlusion

and then lowered in discrete steps of 10 to 20 mm Hg from a starting pressure near 140 mm Hg down to 90 mm Hg while RBF changes were noted on a recorder (Gould). Arterial pressure was held at each level for around 3 min. The systemic arterial pressure was elevated by bilateral occlusion of the carotid arteries. Zero baseline for RBF measurement was obtained by occluding the renal artery distal to the flow transducer after the completion of the flow measurements. These experiments were conducted in Sprague-Dawley rats; the nephritic animals had bilateral disease.

Tubuloglomerular feedback (Sprague-Dawley rats, bilateral NSN)

A group of animals separate from those in the whole kidney studies was used. The left kidney was isolated by a flank incision and was placed in a cup (Lucite®). The microperfusion techniques for characterizing the tubuloglomerular feedback response were essentially the same as those described previously by Schnermann, Persson, and Agerup [14]. Late proximal tubular segments were perfused with an artificial solution composed of 136 mM NaCl, 4 mM $NaHCO_3$, 4 mM KCl, 2 mM CaCl 2, 7.5 mM urea, and 0.1% lissamine green. An 8-μ tip pipette filled with the perfusate was attached to a microperfusion pump (Hampel, Frankfurt, Germany) and with the perfusion rate set at 10 nl/min, a nephron with several surface loops was identified. The perfusion pipette was then withdrawn and reinserted into the last accessible proximal loop. Either vaseline stained with Sudan black (for stop-flow pressure measurement) or bone wax (for early proximal flow rate measurement) was injected into the middle surface proximal loop with a 10 to 15 μ tip pipette. Stop-flow pressure measurements were obtained in segments proximal to the vaseline block using continuous recording servo-nulling micropipette transducer techniques. Micropipettes with 1 to 3 μ tips filled with 1.5 M NaCl were used. The late proximal perfusion rate was alternately set at 15, 20, 30, or 40 nl/min in a random fashion with a reference perfusion rate at 10 nl/min while stop-flow pressures in early proximal tubules were recorded at each setting. In a separate group of animals, exactly timed tubular fluid collections were made in segments proximal to the bone wax block using 8-μ pipettes filled with stained paraffin oil in conjunction with alternating late proximal perfusion rate of 0, 10, 25, or 40 nl/min in a random fashion. A 3-min equilibration period was allowed between pump rate changes before starting either stop-flow pressure measurement or timed collection of tubular fluid. These experiments were performed on Sprague-Dawley rats; the nephritic animals had bilateral disease.

Late proximal flow rate (Sprague-Dawley rats, bilateral NSN)

A separate group of animals served for this study. A jugular vein was catheterized for the injection of filtered 1% FDC and Green dye in isotonic saline. Surgical preparation was made as previously described for tubuloglomerular feedback study. A bolus of 0.05 ml of FDC and Green solution was injected and the passage of the dye through surface tubules was observed. The last accessible superficial proximal loops were identified, and timed tubular fluid collections were made in the last segment using 8-μ pipettes filled with stained paraffin oil. These experiments were performed on Sprague-Dawley rats; the nephritic animals had bilateral disease.

Table 1. Changes in extracellular fluid volume (ECFV) and whole kidney GFR in bilateral NSN, unilateral NSN, and control rats before (B) and after (A) immunization; values are expressed as mean ± SEM

		ECFV *ml/100 g*		GFR *ml/min*	
	N	B	A	B	A
Control rats	10	28.7 ± 0.5	28.8 ± 0.9	1.2 ± 0.1	1.4 ± 0.2
Bilateral NSN	8	26.6 ± 0.9	30.1 ± 0.6[a]	1.3 ± 0.1	1.4 ± 0.1
Unilateral NSN	8	27.1 ± 0.7	27.7 ± 0.7	1.2 ± 0.1	1.3 ± 0.1

[a] $P < 0.02$

Pathologic observations

Light microscopy. Mid-coronal slices of both kidneys were fixed in 10% neutral buffered formalin and sectioned at 4 to 6 μ after being embedded in paraffin. Hematoxylin- and eosin-stained sections were evaluated after slides were coded and randomized. The severity of acute proliferative glomerular lesions were graded 0 to 4+ on an arbitrary semi-quantitative scale. Rats receiving intravenous anti-GBM Ab and revealing lesions less than or equal to 1+ in either kidney were excluded from analysis, as were rats in the unilateral NSN group which revealed less than or equal to 1+ lesions in the left (perfused) kidney or greater than or equal to 1+ lesions in the right (nonperfused) kidney.

Immunofluorescence. A mid-coronal section of each kidney was snap-frozen in isopentane, precooled in liquid nitrogen, and 4 to 6 μ frozen sections were obtained using cryomicrotome (Mills Tissue Tech II, Miles Scientific, Naperville, Illinois). After fixation in ether-alcohol the sections were reacted with fluorescein conjugated goat anti-rabbit IgG, rabbit anti-rat IgG, and rabbit anti-rat C3 (Cappel Laboratories, Cochranville, Pennsylvania). Slides were interpreted without knowledge of the experimental group. Rats receiving intravenous anti-rat GBM Ab which revealed negative reactions for rabbit or rat IgG along GBM were excluded from further analysis. Rats in the unilateral-NSN group which revealed positive reactions for rabbit or rat IgG along GBM of the right (nonperfused) kidney or negative reactions for rabbit or rat IgG along GBM of the left (perfused) kidney were also excluded from further analysis.

Results

Extracellular fluid volume

The results of measurements of whole kidney GFR and ECFV are presented in Table 1. GFR did not change significantly in nephritic rats with either unilateral or bilateral disease, or in sham control rats. GFR values obtained in this set of experiments with a single injection technique were about 60% of those obtained with conventional inulin clearance methods. We found a consistent correlation between the two methods in both normal and diseased animals (Harris and Glassock, unpublished observations). We used the single injection method here because it permits serial determinations on the same animal. The results are presented to show that GFR did not differ among the groups.

Measurements of ECFV indicate that animals with bilateral NSN had a consistent increase, compared to sham control rats.

Table 2. Summary of glomerular measurement[a]

	N	$A\bar{P}$	P_{GC}	P_T	$\Delta\bar{P}$	P_C	Π_A	Π_E	$\Pi_E/\Delta\bar{P}$	SNGFR	SNFF
		mmHg								nl/min	
Normal, hydropenia	6	109.5 ±4.1	42.5[b] ±1.0	12.9 ±0.4	29.6[b] ±0.8	16.5 ±0.5	20.9 ±0.6	33.8 ±0.6	1.15[b] ±0.05	18.7 ±0.7	0.26 ±0.01
Bilateral NSN, hydropenia	12	122.2 ±3.6	49.2 ±1.5	13.5 ±0.3	35.7 ±0.8	16.1 ±0.3	20.1 ±0.8	30.1 ±1.8	0.86 ±0.06	25.3 2.2	0.22 ±0.02
Unilateral NSN, hydropenia	8	109.3 ±4.9	48.1 ±0.9	11.6[b] ±0.4	36.5 ±1.0	14.8[b] ±0.3	21.3 ±0.4	32.1 ±0.6	0.88 ±0.02	23.6 ±2.6	0.23 ±0.01
ANOVA[d]		NS	$P < 0.025$	$P < 0.0025$	$P < 0.025$	$P < 0.025$	NS	NS	$P < 0.005$	NS	NS

[a] Values listed are means ± SEM.

[b] The value indicated differs significantly from the other two, as judged by a Newman-Keuls multiple range test. In no instance did all three means differ from each other.

[c] The Kf value for the normal group was taken from the plasma-loaded normal group. All other measurements in the row labelled "Normal" are from hydropenic animals.

[d] Probabilities listed in the row labelled ANOVA are the probabilities that all means in a given column are the same, as tested by one-way analysis of variance.

This increase is the abnormality the effect of which we wish to examine. The results show no increase in ECFV in unilateral disease. Thus, the unilateral model is one with nephritis but without expansion of ECFV. We have used a comparison of the two models to determine whether the measured ECFV expansion of bilateral NSN is required for the hemodynamic adaptation to the disease.

Glomerular hemodynamics in normal animals

Hydropenic normal rats. Because this study involved some nephritic rats that were in a state of volume expansion caused by the disease, no attempt was made to correct the extravascular shift in extracellular fluid volume known to be caused by laparotomy [15]. The results reflect this effect. Glomerular capillary hydraulic pressure, SNGFR, SNGPF, and SNGBF all tended to be at the lower end of the range of values published for normal animals [16] while hematocrit values were high. Since filtration pressure equilibrium prevailed, exact values for Kf could not be estimated, but the calculated minimum values averaged 0.082 ± 0.006 nl × sec^{-1} × mm Hg^{-1}, a value not different from that reported for normal Wistar-Munich rats [16].

Plasma volume-expanded normal rats. The purpose of these measurements was to provide a reference value of Kf against which the value for nephritic animals could be compared (Table 2). The mean value 0.069 ± 0.012 nl/sec^{-1} mm Hg^{-1} does not differ from the value of 0.078 ± 0.003 nl/sec^{-1} mm Hg^{-1} reported by Deen et al [16] for this strain of Wistar Munich rats.

Glomerular hemodynamics in nephrotoxic serum nephritis (NSN)

Bilateral NSN. Compared to hydropenic control rats, these animals had significant elevations of P_{GC} and $\Delta\bar{P}$ and were not at filtration pressure equilibrium (Table 2). Kf was significantly reduced from normal for this population. Since SNGFR remained unchanged from normal, our results indicate that an elevation of net ultrafiltration pressure compensates for the reduction in Kf to maintain normal SNGFR. Similar results and conclusions have been presented earlier [3]. Glomerular blood flow rate was increased in this group, but $A\bar{P}$ was unchanged; total renal vascular resistance therefore decreased. On the assumption that renal vascular resistance resides primarily in the afferent and efferent arterioles, and given that P_{GC} increased while overall vascular resistance decreased, it follows that there must have been a reduction of afferent arteriolar resistance. The results of measurements of arteriolar resistances tend to bear out this analysis, but the variances are large and the differences do not achieve statistical significance.

Unilateral sham control rats. These groups were designed to serve as a control for the surgical procedure imposed on the unilateral nephritic group. These animals had normal P_{GC} (42.2 ± 0.2 mm Hg) and $\Delta\bar{P}$ (31.9 ± 0.1 mm Hg), and all were at filtration pressure equilibrium ($\pi_E/\Delta\bar{P}$ = 1.15 ± 0.6). None of these values were significantly different from those found in hydropenic control rats.

Unilateral NSN. In all measures except one, these animals exhibited mean values that were not significantly different from those for the bilateral NSN group (Table 2). The single exception was a difference in P_T of 1.9 mm Hg; the unilateral group was lower. This slight difference was too small to cause a significant difference in $\Delta\bar{P}$, however. The unilateral NSN animals had an elevation of P_{GC}, $\Delta\bar{P}$, a reduction in Kf, and unchanged SNGFR. Just as in the bilateral NSN group, the elevation of net ultrafiltration pressure compensated for the reduction in Kf to maintain normal SNGFR. This compensation occurred despite the presence of a normally functioning right kidney and normal ECFV (see **Results**). This result suggests that the stimulus for dilatation is largely intrarenal and does not necessarily involve an extrarenal pathway.

Unilateral NSN—two kidney comparisons

As a further test of the conclusion that the signal mediating the compensation is intrarenal, we measured P_{GC} in both kidneys of unilaterally diseased animals. If there is an extrarenal signal, it should affect both kidneys. Moreover, this comparison avoids interanimal variation as a source of uncertainty and is therefore a more rigorous test of the existence of a difference in P_{GC} than can be obtained from comparisons of one

Table 2. *(Continued)*

SNGPF$_A$ *nl/min*	R_A	R_E	K_F
	10^{10} *dyn · sec · cm^{-5}*		*nl · sec^{-1} · mm Hg^{-1}*
72.7 ±5.4	3.24 ±0.3	1.48 ±0.07	0.069[b,c] ±0.012
144.1 ±30.1	2.79 ±0.5	1.40 ±0.22	0.037 ±0.005
122.5 ±24.6	2.40 ±0.29	1.28 ±0.17	0.043 ±0.006
NS	NS	NS	$P < 0.025$

group of animals with another. As can be seen in Table 3, the diseased kidneys had significantly higher values of P_{GC} than the nonperfused kidney, and P_{GC} was not different in the two kidneys of a sham-perfused group. Thus, the elevation of P_{GC} in the diseased kidney appears to result primarily from a response generated within the organ itself, and not to a systemic signal.

Whole kidney autoregulation in NSN

Figure 1 shows the BP-GFR autoregulation curve in control and bilateral NSN rats. Although GFR was lower in NSN than in control at both high and low ranges of perfusion pressure (1.17 ± 0.08 in NSN vs. 0.75 ± 0.06 ml/min/g of kidney weight in control at high BP, $P < 0.001$; 0.98 ± 0.06 vs. 0.71 ± 0.06 at low BP, $P < 0.01$), the mean slopes of the regression lines were not statistically different from each other, indicating NSN rats autoregulate GFR as well as control rats.

As shown in Figure 2 RBF is as well autoregulated in rats with NSN as in control rats over a range of renal perfusion pressures from 133 to 93 mm Hg. The mean slopes of the regression lines were not different from each other. RBF averaged 8.51 ± 0.89 ml/min/g of kidney weight in control animals and 9.21 ± 1.03 ml/min/g of kidney weight in rats with NSN. The difference was not significant.

Tubuloglomerular feedback in NSN

Stop-flow pressure (SFP) in early proximal tubules was higher in NSN than in control rats by 4.0 mm Hg, at a perfusion rate of 10 nl/min, and remained higher at higher perfusion rates (Fig. 3). This difference corresponds to the difference found by direct micropuncture in Wistar Munich rats (Table 2). The data points from each group of animals were fit to a curve of the form $y = a \exp(bx)$. The values of the coefficient b were: control, $-5.8 \times 10^{-3} \pm 2.2 \times 10^{-3}$; NSN, $-8.4 \times 10^{-3} \pm 2.1 \times 10^{-3}$. There was no significant difference between the calculated values of the two coefficients. Thus, apart from the higher absolute value of SFP, these results suggest that the sensitivity of SFP to the loop of Henle perfusion rate is the same in NSN as in control rats. Early proximal flow rate, an approximation of $SNGFR_{\zeta}$ responded to changes in the loop of Henle perfusion rate in rats with NSN as it did in control rats (Fig. 4).

Finally, we measured the late proximal flow rate to determine whether the signal to tubuloglomerular feedback might be altered in NSN. Late proximal flow rate was significantly higher in rats with NSN than in control rats, indicating that the macula densa receives a signal that should cause a lower level of GFR than in control rats (Table 4).

Table 3. Comparison of glomerular capillary pressures (P_{GC}) in right and left kidneys of unilaterally diseased and unilaterally sham-operated rats; operative procedures were performed on the left kidneys

	Sham ($N = 6$)		NSN ($N = 8$)	
	Left	Right	Left	Right
P_{GC}, *mm Hg*	48.0 ± 3.9	45.3 ± 3.0	56.4 ± 1.5	50.4 ± 1.8
Paired difference, *mm Hg*	2.75 ± 1.5		6.0 ± 1.5	
	$0.1 < P < 0.05$		$P < 0.001$	

Pathology and immunopathology

Bilateral NSN. The left and right kidneys of rats in the bilateral NSN group displayed mild to moderate (2 to 3+) acute proliferative glomerulonephritic lesions consisting of infiltration with one to five polymorphonuclear leucocytes and/or monocytes. The glomeruli were hypercellular and the capillary lumens were segmentally obliterated. Necrosis and crescent formation were not observed. The tubules, extraglomerular vasculature and interstitium, were normal. The lesions were uniform throughout the superficial and deep cortex. Intense diffuse linear deposits of rabbit IgG were seen in all glomeruli, but no deposits were seen in tubular basement membranes or extraglomerular vessels. Diffuse glomerular deposits of rat IgG and C3 were seen in all rats, although the pattern tended to be less linear and more segmental. These findings are similar to those reported previously [3].

Unilateral NSN. Mild to moderate proliferative lesions (2+ to 3+) similar in character to that observed in bilateral NSN were seen in the left (perfused) kidney of unilateral NSN group, whereas the right (nonperfused) kidneys could not be distinguished from normal (Fig. 5). Diffuse linear deposits of rabbit IgG and to a lesser extent rat IgG in C3 were found in the left (perfused) kidney, while the right kidneys showed only scattered, trace deposits of rabbit IgG or rat IgG/C3 (Fig. 6).

Control rats. The glomeruli in the normal hydropenic, plasma-loaded, unilateral-sham control groups were uniformly normal by light microscopy and negative by immunofluorescence for deposits of rabbit IgG, rat IgG, or rat C3.

Discussion

The results of this study confirm earlier findings in the early autologous phase of NSN [3] in that a significant reduction of Kf can be tolerated without a fall in SNGFR because of a compensating increase in net ultrafiltration pressure (P_{UF}). Of the three components of P_{UF}, the principal change is an elevation of P_{GC}. The compensation appears to be quite precise, because SNGFR does not decline despite a substantial reduction of Kf. This precision suggests that a rather vigorous control mechanism is at work. The identification of this mechanism is the goal of this study.

Although SNGFR can be maintained at normal levels despite a reduction in Kf to 30 to 50% of normal and an increase in end proximal flow rate (present results and [4]), we found that ECFV is increased in bilateral NSN. The response of the

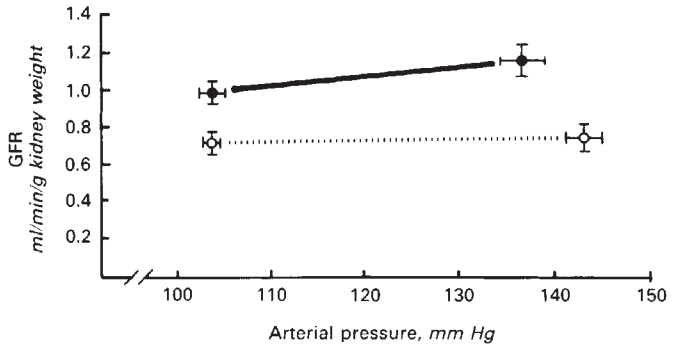

Fig. 1. *Whole kidney GFR as a function of arterial pressure.* Symbols are: ○ . . . . ○, rats with nephrotoxic serum nephritis; ● —— ●, control rats.

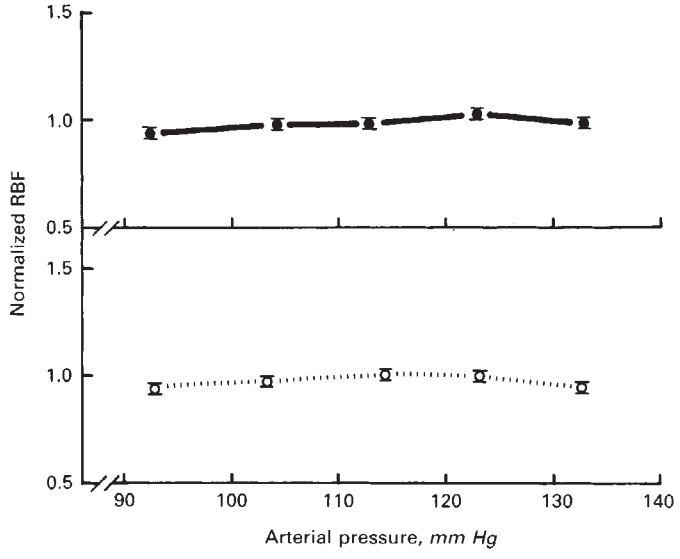

Fig. 2. *Whole kidney blood flow as a function of arterial pressure in rats with and without nephrotoxic serum nephritis.* Blood flows were normalized by the flow at the ambient arterial pressure before the pressure was raised by carotid occlusion. Symbols are the same as those used in Figure 1.

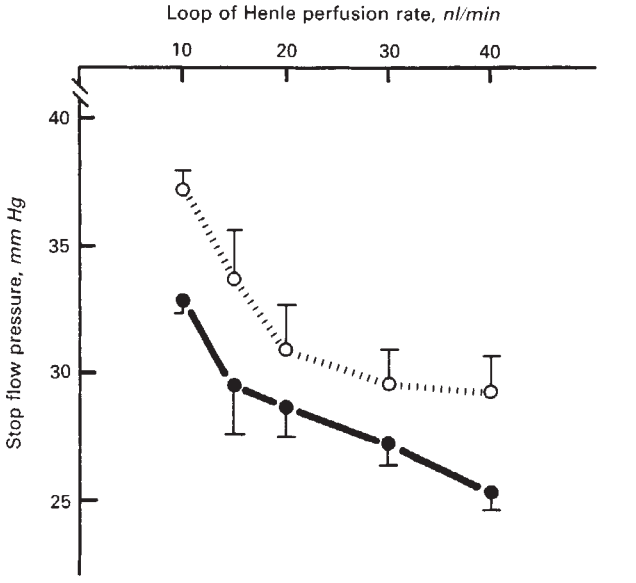

Fig. 3. *The change in proximal stop-flow pressure as a function of loop of Henle perfusion rate.* Symbols are the same as those used in Figure 1.

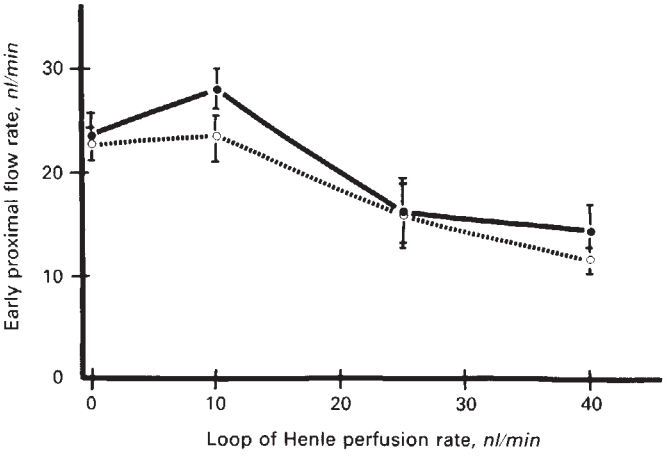

Fig. 4. *Early proximal flow rate as a function of loop of Henle perfusion rate.* Symbols are the same as those used in Figure 1.

systems mediating blood flow and GFR autoregulation are known to be altered by changes in extracellular fluid volume [5, 6]. To identify the mechanism responsible for the adaptation of NSN, it becomes important to decide whether the volume expansion is a major factor in the response. The unilateral NSN model was designed to address this question. With unilateral disease, both the normal and the diseased kidney are exposed to the same signal arising from a given perturbation of extracellular fluid volume. If both kidneys experience the same elevation of P_{UF}, volume expansion might be a major factor; if the difference between the two kidneys remains, an intrarenal mechanism will have to be identified. The results of Kf measurements show that with the unilateral model of NSN a lesion of comparable severity to that induced with the more routine bilateral approach was obtained. Direct comparisons of P_{GC} in the same animal revealed a difference in each animal as great as was found between the bilaterally diseased and normal animals, although ECFV was normal. This comparison was made in anesthetized animals subjected to animal surgery, while the ECF volume measurements were made in conscious animals. If the results of the direct P_{GC} are applicable to conscious animals, it would follow that an intrarenal mechanism plays a major role in the renal adaptation to moderate NSN.

We next assessed several measures of autoregulation in

Table 4. Comparison of late proximal tubule flow rate in rats with and without bilateral NSN

	Control rats	NSN
Number of animals	6	7
Number of tubules	38	39
Flow rate, *nl/min*	11.8 ± 1.18	16.3 ± 1.0
	$P < 0.01$	

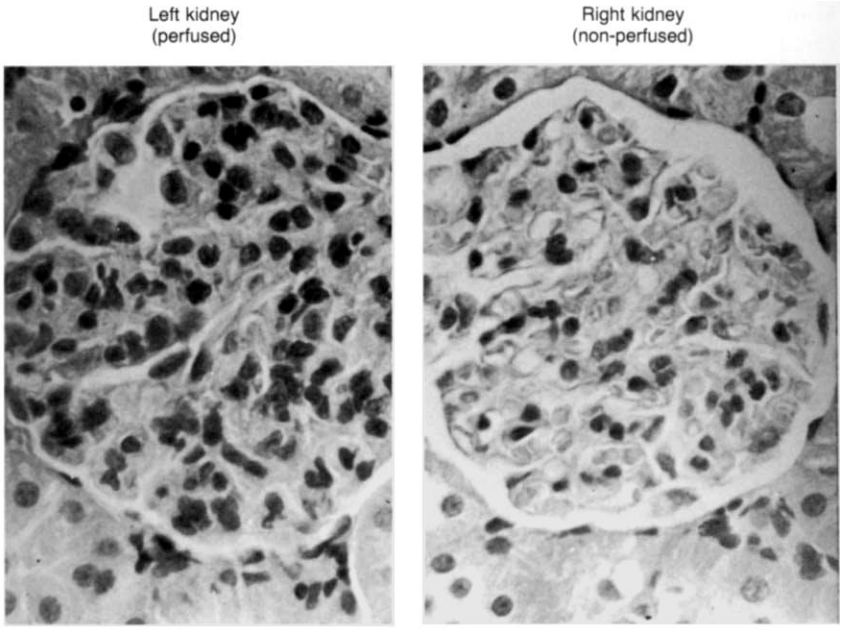

Fig. 5. *A comparison of the light microscopic changes in the left* (perfused) *and right* (nonperfused) *kidneys of a rat with unilateral nephrotoxic serum nephritis.* (14 days postinjection of anti-GBM antibody, hematoxylin and eosin, ×250)

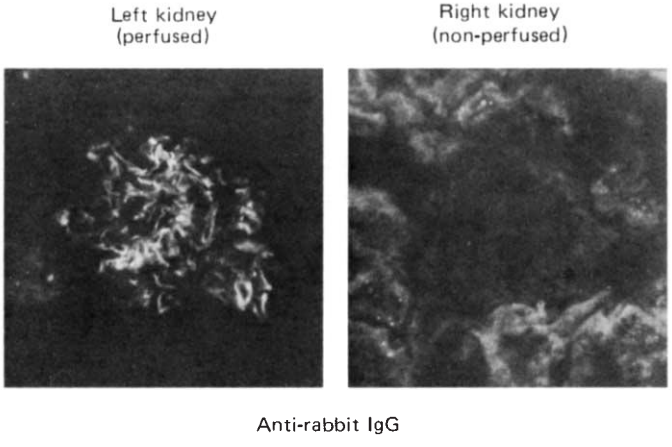

Fig. 6. *A comparison of the deposition of rabbit IgG anti-GBM antibody in the left* (perfused) *and right* (nonperfused) *kidneys of a rat with unilateral NSN.* (14 days, postinjection of anti-GBM antibody, immunofluorescence with fluorescein-labeled goat anti-rabbit IgG, ×200)

NSN. The two mechanisms thought to be of principal importance are a myogenic mechanism and tubuloglomerular feedback mediated via the macula densa. Stop-flow pressure is a measure of P_{GC}, but differs from it because the suppression of filtration that is a necessary part of the determination increases efferent arteriolar plasma flow and P_{GC} [17]. Changes in stop-flow pressure probably mirror changes in P_{GC}. With the loop of Henle perfusion set at 10 nl/min, stop-flow pressure was higher in rats with NSN than in control rats, by an amount comparable to the difference in P_{GC} between NSN and normal rats. The response of stop-flow pressure and early proximal flow rate to changes in loop of Henle perfusion rate was not detectably different than the response of control animals. We conclude that immune glomerular injury does not alter the performance of tubuloglomerular feedback in a way that could explain the afferent arteriolar dilatation that is presumed to be a major adaptive response to NSN. Since our results show that the disease causes a mild expansion of ECFV, the fact that the feedback response has not been attenuated suggests either that the expansion is too mild to produce a measurable effect on the measures of feedback we have used, or that the feedback mechanism has developed an increased sensitivity that compensates for the effect of expansion. We have not attempted to determine which of these explanations is correct.

If the response of this control mechanism to isolated testing is not abnormal, feedback could cause afferent arteriolar dilatation only if the macula densa received a reduced flow signal. Instead as we and Maddox et al [4] have shown, the flow rate of tubular fluid into the loop of Henle is increased because of reduced fluid reabsorption in the proximal tubule. Finally, with tubuloglomerular feedback in a normal functional state, and with whole kidney autoregulation also normal, it follows that there can be no major abnormality in the myogenic mechanism.

Our studies suggest that some possible causes of the adaptation are unlikely but have not further identified the mechanism. As we pointed out previously [18], mechanisms like the myogenic response and tubuloglomerular feedback have time constants of response that are measured in seconds and tenths of seconds. In retrospect it is perhaps not surprising that the response to a slowly developing lesion should involve mechanisms different from these fast reacting controllers. Our current working hypothesis is that the stimulus to adaptation induces slow changes in the afferent arteriole, possibly structural, and that mechanisms responsible for autoregulation adapt sufficiently and continue to maintain blood flow and GFR constant in response to higher frequency perturbations.

The glomerular hemodynamic response to other forms of experimental injury does not reveal a consistent pattern of response and so does not help to identify possible adaptive mechanisms operating in NSN. Bohrer et al [19] have analyzed glomerular hemodynamics in acute (8-day) aminonucleoside of puromycin-induced nephrosis in rats. Both GPF and Kf were reduced compared to control rats. Interestingly, $\Delta\overline{P}$ was unchanged in the nephrotic as compared to the control rats. These

findings would be most compatible with an increase in renal vascular resistance shared equally by the afferent and efferent arterioles. Baylis, Rennke, and Brenner [20] examined the response to acute (11-day) gentamicin nephrotoxicity in rats. The pattern of disturbance in glomerular hemodynamics observed in this model was quite similar to that seen in the aminonucleoside of puromycin model. Thus, these two experimental models of glomerular injury, both of which result in reductions in Kf comparable to that observed in NSN are not accompanied by a reduction in renal vascular resistance but rather are accompanied by an increase in both R_A and R_E.

On the other hand, Ichikawa et al [21] recently examined two experimental models of a nonproliferative immune complex mediated membranous glomerulopathy; namely, heterologous and autologous immune complex nephritis. In these models Kf was reduced to an equal extent and was not different from that seen in the model of NSN utilized in the present study. $\Delta\overline{P}$ was increased in both models, partially offsetting the effect of reduced Kf on SNGFR; however, GPF was either unchanged or decreased indicating a degree of increased efferent resistance with a minor change in afferent resistance. Taken together, these findings indicate that the hemodynamic response to acute glomerular injury depends on the method of induction of injury, rather than being a generalized response to a reduction in Kf. The failure of $\Delta\overline{P}$ to rise in conjunction with the reduced Kf in aminonucleoside of puromycin nephrosis and gentamicin nephrotoxicity contributes to the observed fall in SNGFR. The increase in $\Delta\overline{P}$ seen in heterologous and autologous immune complex glomerulonephritis and nephrotoxic serum nephritis offsets the reduction in Kf and permits maintenance of a normal or near normal SNGFR. The failure of a single pattern of response to emerge means that each model will have to be pursued independently to determine the cause of adaptation.

Acknowledgments

This work was supported by National Institutes of Health grants AM16565 and AM15968 and by Investigator Group Award 536IG from the American Heart Association, Greater Los Angeles Affiliate. Dr. F. H. Harris, Jr., was supported by a Fellowship Award also from the American Heart Association, Greater Los Angeles Affiliate.

Reprint requests to Dr. R. J. Glassock, Department of Medicine, Harbor-UCLA Medical Center, 1000 W. Carson St., Torrance, California 90509, USA

References

1. Rocha A, Marcondes M, Malnic A: Micropuncture study in rats with experimental glomerulonephritis. *Kidney Int* 3:356, 1974
2. Allison MEM, Wilson CB, Gottschalk CW: Pathophysiology of experimental glomerulonephritis in rats. *J Clin Invest* 53:1402, 1974
3. Maddox DA, Bennett CM, Deen WM, Glassock RJ, Knutson D, Daugharty TM, Brenner BM: Determinants of glomerular filtration in experimental glomerulonephritis in the rat. *J Clin Invest* 55:305, 1975
4. Maddox DA, Bennett CM, Deen WM, Glassock RJ, Brenner BM: Control of proximal tubule fluid reabsorption in experimental glomerulonephritis. *J Clin Invest* 55:1315, 1975
5. Bennett CM, Glassock RJ: Exchangeable sodium in acute nephrotoxic serum nephritis in rats, in *VII Int Cong Nephrol*, 1978, abstracts, K-15
6. Schnermann J, Hermle M, Schmidmeier E, Dahlheim H: Impaired potency for feedback regulation of glomerular filtration rate in DOCA escaped rats. *Pfluegers Arch* 358:325–338, 1975
7. Ploth DW, Rudolph J, Thomas C, Navar LG: Renal and tubuloglomerular feedback responses to plasma expansion in the rat. *Am J Physiol* 235:F156–F162, 1978
8. Hoyer JH, Mauer SM, Michael AF: Unilateral renal disease in the rat. I. Clinical, morphologic and glomerular mesangial functional features of the experimental model produced by renal perfusion with aminonucleoside. *J Lab Clin Med* 85:756–768, 1975
9. Lerner RA, Dixon FJ: Transfer of ovine experimental allergic glomerulonephritis (EAG) with serum. *J Exp Med* 124:431–442, 1966
10. Landis EM, Pappenheimer JR: Exchange of substances through capillary walls. *Handbook Physiol* 2:961–1034, 1963
11. Vurek GG, Pegram SE: Flurometric method for the determination of nanogram quantities of insulin. *Anal Biochem* 16:404–419, 1966
12. Führ J, Kaczmarczyk J, Krüttgen CD: Eine einfache colorimetrische methode zur Inulin Bestimung für Nieren-Clearanceuntersuchungen bei Stoffwechselgesunden und diabetikern. *Klin Wochenschr* 33:729–730, 1955
13. Lowry AH, Rosebrough NF, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–267, 1951
14. Schnermann J, Persson AEG, Agerup B: Tubuloglomerular feedback: nonlinear relation between glomerular hydrostatic pressure and loop of Henle perfusion rate. *J Clin Invest* 52:862–869, 1973
15. Maddox DA, Price DC, Rector FC Jr: Effects of surgery on plasma volume and salt and water excretion in rats. *Am J Physiol* 233:F600–F606, 1977
16. Deen WM, Troy JL, Robertson CR, Brenner BM: Dynamics of glomerular ultrafiltration in the rat. IV. Determination of the ultrafiltration coefficient. *J Clin Invest* 52:1500–1508, 1973
17. Ichikawa I: Evidence for altered glomerular hemodynamics during acute nephron obstruction. *Am J Physiol* 242:F580–F585, 1982
18. Marsh DJ: Frequency response of autoregulation. *Kidney Int* 22:S165–S172, 1982
19. Bohrer MP, Baylis C, Robertson CR, Brenner BM: Mechanism of the puromycin-induced defects in the transglomerular passage of water and macromolecules. *J Clin Invest* 60:152–161, 1977
20. Baylis C, Rennke HR, Brenner BW: Mechanism of the defect in glomerular ultrafiltration associated with gentamicin administration. *Kidney Int* 12:344–353, 1977
21. Ichikawa I, Hoyer JR, Seiler MW, Brenner BM: Mechanism of glomerulotubular balance in the setting of heterogenous glomerular injury-preservation of a close functional linkage between individual nephrons and surrounding microvasculature. *J Clin Invest* 69:185–198, 1982